PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/82, 15/53, A01H 5/00

A2

(11) International Publication Number: **WO 00/20615**

(43) International Publication Date: 13 April 2000 (13.04.00)

(21) International Application Number: PCT/US99/23256

(22) International Filing Date: 5 October 1999 (05.10.99)

(30) Priority Data:
60/103,031 5 October 1998 (05.10.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/103,301 (CIP)
Filed on 5 October 1998 (05.10.98)

(71) Applicant *(for all designated States except US)*: PRODIGENE, INC. [US/US]; Suite 101, 101 Gateway Boulevard, College Station, TX 77845 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: HOOD, Elizabeth [US/US]; 8605 Amber Hill Court, College Station, TX 77845 (US). HOWARD, John [US/US]; 5819 Stallion Ridge, College Station, TX 77845 (US). JILKA, Joseph [US/US]; 2308 Ferguson Street, College Station, TX 77845 (US).

(74) Agent: SWEENEY, Patricia, A.; 1835 Pleasant Street, West Des Moines, IA 50265–2334 (US).

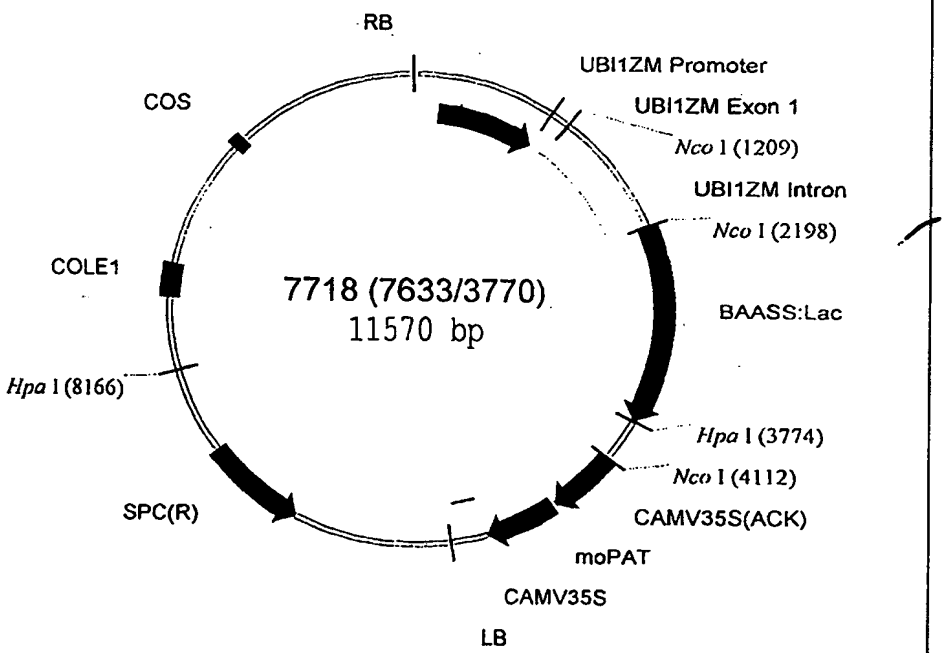
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: COMMERCIAL PRODUCTION OF LACCASE IN PLANTS

(57) Abstract

Expression of laccase in plants at commercial levels of production is provided. The laccase gene vectors may include signal sequences directing expression to the cell wall of the plant, and may additionally include sequences targeting expression to the endoplasmic reticulum of the plant cell. Expression of laccase may also be directed to the seed of the plant. The plant having the laccase gene may additionally contain a substance that is a mediator of laccase delignification. Methods of improving the process of introducing DNA into plants via *Agrobacterium* are also provided.



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PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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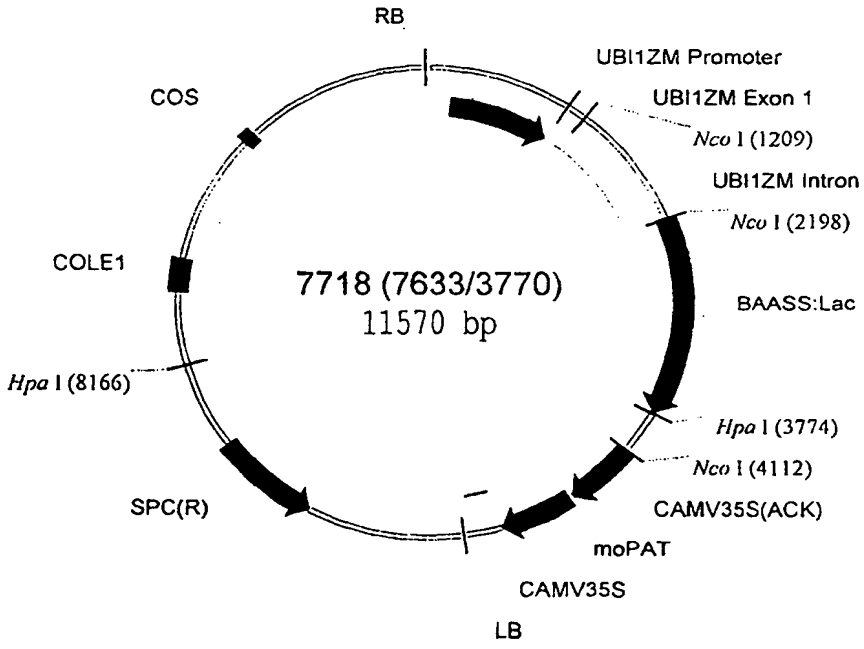
Published
With international search report.

(88) Date of publication of the international search report:
16 November 2000 (16.11.00)

(54) Title: COMMERCIAL PRODUCTION OF LACCASE IN PLANTS

(57) Abstract

Expression of laccase in plants at commercial levels of production is provided. The laccase gene vectors may include signal sequences directing expression to the cell wall of the plant, and may additionally include sequences targeting expression to the endoplasmic reticulum of the plant cell. Expression of laccase may also be directed to the seed of the plant. The plant having the laccase gene may additionally contain a substance that is a mediator of laccase delignification. Methods of improving the process of introducing DNA into plants via *Agrobacterium* are also provided.



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INTERNATIONAL SEARCH REPORT

Internat' ıl Application No
PCT/US 99/23256

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/53 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 11205 A (FLETCHER CHALLENGE FORESTS LIM ;GENESIS RESEARCH & DEV CORP LI (NZ) 19 March 1998 (1998-03-19) cited in the application page 3; example 7; table 3 ---	1-4,11, 13-15
X	WO 97 45549 A (CENTRE NAT RECH SCIENT ;FAYE LOIC (FR); GOMORD VERONIQUE MARTINE () 4 December 1997 (1997-12-04) cited in the application the whole document ---	1-4,11, 13-15
Y	WO 97 09431 A (NOVO NORDISK BIOTECH INC) 13 March 1997 (1997-03-13) page 4 -page 9 --- -/--	1-4,7, 9-11, 13-15, 20-26

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 March 2000	25. 07. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 693 506 A (RODRIGUEZ RAYMOND L) 2 December 1997 (1997-12-02) column 8, line 50 - line 64	1-11, 13-15, 18,20-26
Y	US 5 770 418 A (AOLYNG DORRIT A ET AL) 23 June 1998 (1998-06-23) the whole document	1-11, 13-15, 18,20-26
Y	ONG E ET AL: "Cloning and sequence analysis of two laccase complementary DNAs from the ligninolytic basidiomycete Trametes versicolor" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES,GB,ELSEVIER SCIENCE PUBLISHERS, BARKING, vol. 196, no. 1-2, 1 September 1997 (1997-09-01), pages 113-119, XP004126336 ISSN: 0378-1119 the whole document	1-11, 13-15, 18,20-26
A	CRESTINI C ET AL: "THE EARLY BIODEGRADATION PATHWAYS OF RESIDUAL KRAFT LIGNIN MODEL COMPOUNDS WITH LACCASE" ISWPC,1997, pages 1-5, XP000863181 the whole document	
A	BOURBONNAIS R ET AL: "ENZYMATIC DELIGNIFICATION OF KRAFT PULP USING LACCASE AND A MEDIATOR" TAPPI JOURNAL,US,TECHNICAL ASSOCIATION OF THE PULP & PAPER INDUSTRY. ATLANTA, vol. 79, no. 6, 1 June 1996 (1996-06-01), pages 199-204, XP000640089 ISSN: 0734-1415 the whole document	
P,X	DE 197 52 666 A (IPK INST FUER PFLANZENGENETIK) 1 July 1999 (1999-07-01) the whole document	1-4, 15-17
E	WO 00 05381 A (CALGENE LLC ;SCHAAF DAVID J (US); STALKER DAVID M (US); THOMAS STE) 3 February 2000 (2000-02-03) page 4; claim 9	1-11, 13-15, 18,20-26

INTERNATIONAL SEARCH REPORT

Int. tional application No.
PCT/US 99/23256

Box I Observations where certain claims were found unsearchable (C ntinuation f item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-26 completely

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-26 completely

A transgenic plant expressing a Trametes versicolor-specific laccase as specified by SEQID 1; furthermore a method for producing said laccase in commercial quantities followed by extraction of the laccase from said plant.

2. Claims: 27-31 completely

A method for transforming a plant with a gene of interest using an Agrobacterium strain comprising a cointegrated superbinary/cloning vector.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/US 99/23256

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9811205 A	19-03-1998	US 5850020 A	15-12-1998
		AU 4403697 A	02-04-1998
		BR 9711745 A	18-01-2000
		EP 0929682 A	21-07-1999
		US 5952486 A	14-09-1999
		ZA 9710451 A	20-05-1999
WO 9745549 A	04-12-1997	FR 2749322 A	05-12-1997
		AU 3097297 A	05-01-1998
WO 9709431 A	13-03-1997	AU 7154096 A	27-03-1997
		EP 0850306 A	01-07-1998
US 5693506 A	02-12-1997	AU 703288 B	25-03-1999
		AU 1289295 A	06-06-1995
		CA 2176834 A	26-05-1995
		EP 0788550 A	13-08-1997
		JP 9509565 T	30-09-1997
		WO 9514099 A	26-05-1995
		US 5889189 A	30-03-1999
		US 5994628 A	30-11-1999
		US 5888789 A	30-03-1999
US 5770418 A	23-06-1998	AU 698276 B	29-10-1998
		AU 2827895 A	19-01-1996
		BR 9508113 A	14-07-1998
		CA 2193070 A	04-01-1996
		CN 1157636 A	20-08-1997
		EP 0767836 A	16-04-1997
		FI 965201 A	21-02-1997
		JP 10502806 T	17-03-1998
		NZ 288901 A	27-04-1998
		WO 9600290 A	04-01-1996
		US 5667531 A	16-09-1997
DE 19752666 A	01-07-1999	NONE	
WO 0005381 A	03-02-2000	US 6013860 A	11-01-2000
		EP 1017824 A	12-07-2000

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 22 May 2000 (22.05.00)	
International application No. PCT/US99/23256	**Applicant's or agent's file reference** 1015
International filing date (day/month/year) 05 October 1999 (05.10.99)	**Priority date** (day/month/year) 05 October 1998 (05.10.98)
Applicant HOOD, Elizabeth et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

18 April 2000 (18.04.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Juan Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

1/

1285.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
10015-PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/23256	05/10/1999	05/10/1998
International Patent Classification (IPC) or national classification and IPC C12N15/82		
Applicant PRODIGENE, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/04/2000	13.12.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Trommsdorff, M Telephone No. +49 89 2399 7361

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/23256

I. Basis f the rep rt

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-24 as originally filed

Claims, No.:

1-31 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

Sequence listing part of the description, pages:

1-6, filed with the letter of 7.02.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 3, 4, 27-31.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. 3, 4 are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 27-31.

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-26.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7-12, 16, 17, 20-24
	No:	Claims	1-2, 5, 6, 13-15, 18, 25-26
Inventive step (IS)	Yes:	Claims	12, 19

	No:	Claims	1, 2, 5-11, 13-18, 20-26
Industrial applicability (IA)	Yes:	Claims	1, 2, 5-26
	No:	Claims	3, 4: no opinion

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Cit d docum nts

The following documents (D) are referred to in this communication; the numbering is the same as in the search report and will be adhered to in the rest of the procedure:

D1: WO 98 11205 A (FLETCHER CHALLENGE FORESTS LIM ;GENESIS RESEARCH & DEV CORP LI (NZ) 19 March 1998 (1998-03-19) cited in the application

D2: WO 97 45549 A (CENTRE NAT RECH SCIENT ;FAYE LOIC (FR); GOMORD VERONIQUE MARTINE () 4 December 1997 (1997-12-04) cited in the application

D3: WO 97 09431 A (NOVO NORDISK BIOTECH INC) 13 March 1997 (1997-03-13)

D5: US-A-5 770 418 (AOLYNG DORRIT A ET AL) 23 June 1998 (1998-06-23)

D6: ONG E ET AL: 'Cloning and sequence analysis of two laccase complementary DNAs from the ligninolytic basidiomycete Trametes versicolor' GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES,GB,ELSEVIER SCIENCE PUBLISHERS, BARKING, vol. 196, no. 1-2, 1 September 1997 (1997-09-01), pages 113-119

D10: WO 00 05381 A (CALGENE LLC ;SCHAAF DAVID J (US); STALKER DAVID M (US); THOMAS STE) 3 February 2000 (2000-02-03)

2. Content of the application

The present application describes the commercial production of laccase, an enzyme used in different processes such as, eg. the breakdown of lignin: DNA and protein sequences of said enzyme purified from the white rot fungus *Trametes versicolor*, expression vectors and plants transformed with said vectors are described.

3. Re Item II
Priority

The contents of the description of the priority document and of the application as filed appear to be the same with regard to the subject-matter of claims 1-8, 13-18, 20, 21, 25 and 26. Thus, the priority is valid for said claims.
Claims 9-12, 19 and 22-24 however do not have a valid priority, since the subject-

matter claimed in said claims is neither explicitly nor implicitly contained in the priority document.

4. **Re Item IV**

Lack of unity of invention

The International Search Authority found multiple groups of inventions in this international application. Only the first group of inventions has been searched, i.e. claims 1-26. Accordingly, the examination is also restricted to claims 1-26.

5. **Re Item V**

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

5.1. Novelty of the claims

Methods of production of laccase in fungi are well known in the art. The major laccase sources are white rot fungi but other fungi such as, eg. *Neurospora* or *Aspergillus* are also comonly used.

According to the description, the natural expression in plants is low, i.e. less than 0.001% of the total soluble plant protein (p.2, l.26-27). The object of the invention is to obtain higher yields of laccase in order to reach a commercially acceptable level (p.3, l.2-6).

The solution described by the applicants appears to be the expression of a recombinant laccase driven by a specific promoter such as, eg. the globulin promoter directed to seeds (claims 12 and 19). The applicants show that in transfected maize the expression of said recombinant laccase reaches 0.14% of the total soluble protein of the plant.

The claims do not however represent this finding in an appropriate manner for the following reasons:

Claims 1-4 are directed to a transgenic plant comprising a nucleotide sequence encoding laccase, linked to a promoter, wherein the laccase represents 0.01%,

0.1%, 1% or 10%, respectively of the total soluble protein of the plant.

Several prior art documents disclose the expression of laccases in transgenic plants.
D1 describes the transformation of tobacco plants with DNA sequences encoding the laccase gene isolated from *Pinus radiata* or *Eucalyptus grandis*. The authors show that the over expression of anti-sense or sense sequences of said gene affects the lignin biosynthesis in the transformed plants (examples 5-7, p.21-24, claim 15). The authors further measure enzyme activities of crude extracts (table 4, p.24).
D2 describes the transformation of plants with the cDNA sequence encoding a tobacco laccase (p.16, l.20-p.17, l.12 and p.24, l.7- 35). The authors further suggest the transformation of corn with the laccase gene (p.18, l.10-12).

Thus, the only technical feature of claims 1-4 that allegedly differs from said documents is the expression level of laccase above 0.01%. As discussed previously, the aim of the applicants is to obtain high yields of laccase expression. However, the present authority cannot aggree that 0.01% expression as required by claim 1 corresponds to a high yield of expression, let alone that this yield distinguishes claim 1 from the prior art. Indeed, even though the expression levels of the recombinant laccase are not explicitly mentioned in D1 and D2, one can expect to obtain similar levels as with the recombinant laccase from the application. Unless a difference in expression levels is not experimentally confirmed, this feature cannot establish novelty.
The same applies to dependent claim 2, wherein the expression level claimed is also low, i.e. 0.1%.
Thus, in order to render said claims admissible under Art. 33(1) as well as Art. 6 PCT, the applicants are requested to add in the claims the technical features (a specific promoter such as, eg. the globulin promoter of claim 12, etc...) that are necessary for achieving the higher expression of laccase.

In any case, claims 3 and 4 cannot be allowed, since the expression levels claimed, i.e. 1% or 10%, respectively are much higher than in the description wherein the level of expression never exceeds 0.14%. Furthermore, no experimental procedures are disclosed in the description that would allow the

skilled person to obtain such high expression yields. Thus, the subject-matter of these claims lacks support from the description. Consequently, said claims need to be deleted (Art. 6 PCT).

Dependent claim 5 is directed to the plant of claim 1, wherein the plant is corn. Since D2 suggests the use of corn (p.18, l.10-12), it is novelty destroying for said claim.

Dependent claims 6, 13 and 14 are directed to seeds (claim 13), cells (claim 14) of the plant of claim 1 or the expression of laccase in seeds.
Since the plant claimed in claim 1 lacks novelty, cells or seeds of said plant also lack novelty.

The same reasoning as for claim 1 applies to the method claims 15 and 25.
Claim 15 is directed to a method of producing laccase in plants wherein the laccase is expressed at levels of 0.01% or higher of soluble protein.
Just like claim 1, claim 25 lacks the specific technical features that would lead to said "increased" level of expression.
Thus, claim 15 and dependent claim 18 are not novel.
Claim 25 is directed to a method wherein certain plants contain nucleotidic sequences coding for the laccase and said enzyme is extracted from the plants.
No additional technical feature can be seen in this claim that could render it novel over the prior art documents D1 and D2. The only feature that is not disclosed in D1 (or D2) is the extraction step. However, protein extraction methods are well-known in the art and thus, the extraction of laccase cannot establish novelty of said claim over the prior art.
Claim 26 is directed to the same method, wherein the expression levels are about 0.01%. As previously discussed, this feature alone cannot establish novelty over the prior art.
Hence, claims 25 and 26 also lack novelty.

5.2. Inventive step of the claims

Claim 7 is directed to the plant of claim 1, wherein the laccase is of fungal origin.
D5 describes the expression of the fungal laccase from *P. pinsitus*, also referred

to as *Trametes villosa* in *Aspergillus* (§III., p.21-p.23, l.17). The difference between D3 and claim 7 lies in the choice of the host organism, which is a fungus in D3 and a plant in claim 7. Since the use of a plant as a host is suggested by the authors (p.6, l.5-8) and since both, D1 and D2 teach the transformation of plants with a laccase gene (see above) no inventive step can be seen for the subject-matter of claim 7.
Note, that D3 also shows the transformation of *Aspergillus oryzae* with *Myceliophtora thermophila* laccase genes (example 2, p.15, l.26- p.16, l.7) and suggests the use of a laccase from *Trametes* (p.4, l.10).

Claim 8 is directed to a maize plant transformed with a *Trametes versicolor* laccase encoding sequence. Since none of the prior art describes such a plant, this claim is novel. However, the selection of maize as a host plant among other potential host plants does not appear to be inventive.

Claims 9-11 are directed to the plant of claim 1, wherein the laccase sequence has a certain degree of identity with seq. ID no. 1 of the application, i.e. the laccase cDNA sequence from *Trametes versicolor* (claims 9, 10) or hybridizes to said sequence (claim 11).
As discussed previously, D5 describes the expression of the laccase from *Trametes villosa* in *Aspergillus* (§III., p.21-p.23, l.17). The search alignment shows that the laccases of *T. villosa* and *T. versicolor* are closely related and almost identical over long stretches of sequence (see the annex). Thus, the laccase of D5 fulfills the technical criteria of the laccase of claims 9-11. The difference lies in the host organism. However, D5 further suggests the expression of laccase in plants (p.6, l.5-8). Thus, no inventive step can be seen for claims 9-11.
Note that the nucleic and amino acid sequences of the *T. versicolor* laccase are disclosed in D6 (fig.1, p.114) and thus also available to the skilled person.

Claims 20-24 are method claims that contain technical features corresponding to the product claims 7-11, respectively.
Thus, the same reasoning made for claims 7-11 applies mutatis mutandis to said claims (see § 5.1). Consequently, claim 20-24 lack an inventive step.

Claims 16 and 17 are directed to the method of claim 15 wherein the laccase

expression is specifically targeted to the cell wall (claim 16) or to the endoplasmic reticulum (claim 17). The use of tissue specific promoters or of signal sequences to direct the expression of a given protein to specific cell compartments or tissues is well known in the art. In D9 several prior art documents are cited that disclose signal peptides and tissue specific promoters (see p. 5, l.35-58).
Thus, no inventive step can be seen for the subject-matter of claims 16 and 17.

6. Re Item VI
Certain documents cited (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 00 05381 A	03.02.2000	23.07.1999	24.07.1998

7. Re Item VII
Certain defects in the international application

7.1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D3, D5 and D6, is not mentioned in the description, nor are these documents identified therein.

8. Re Item VIII
Certain observations on the international application

8.1. The relative term "stringent conditions" used in claims 11 and 24 has no well-recognised meaning, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT). Indeed, it is well-known in the art that what is meant by "stringent conditions" can vary from one laboratory to another.

0 S

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rul s 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
1015		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/23256	05/10/1999	05/10/1998
Applicant		
PRODIGENE, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readble form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3: ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ None of the figures.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/23256

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-26 completely

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-26 completely

A transgenic plant expressing a Trametes versicolor-specific laccase as specified by SEQID 1; furthermore a method for producing said laccase in commercial quantities followed by extraction of the laccase from said plant.

2. Claims: 27-31 completely

A method for transforming a plant with a gene of interest using an Agrobacterium strain comprising a cointegrated superbinary/cloning vector.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/23256

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/53 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 11205 A (FLETCHER CHALLENGE FORESTS LIM ;GENESIS RESEARCH & DEV CORP LI (NZ) 19 March 1998 (1998-03-19) cited in the application page 3; example 7; table 3 ---	1-4,11, 13-15
X	WO 97 45549 A (CENTRE NAT RECH SCIENT ;FAYE LOIC (FR); GOMORD VERONIQUE MARTINE () 4 December 1997 (1997-12-04) cited in the application the whole document ---	1-4,11, 13-15
Y	WO 97 09431 A (NOVO NORDISK BIOTECH INC) 13 March 1997 (1997-03-13) page 4 -page 9 --- -/--	1-4,7, 9-11, 13-15, 20-26

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 March 2000	25. 07. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/23256

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 693 506 A (RODRIGUEZ RAYMOND L) 2 December 1997 (1997-12-02) column 8, line 50 - line 64	1-11, 13-15, 18,20-26
Y	US 5 770 418 A (AOLYNG DORRIT A ET AL) 23 June 1998 (1998-06-23) the whole document	1-11, 13-15, 18,20-26
Y	ONG E ET AL: "Cloning and sequence analysis of two laccase complementary DNAs from the ligninolytic basidiomycete Trametes versicolor" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES,GB,ELSEVIER SCIENCE PUBLISHERS, BARKING, vol. 196, no. 1-2, 1 September 1997 (1997-09-01), pages 113-119, XP004126336 ISSN: 0378-1119 the whole document	1-11, 13-15, 18,20-26
A	CRESTINI C ET AL: "THE EARLY BIODEGRADATION PATHWAYS OF RESIDUAL KRAFT LIGNIN MODEL COMPOUNDS WITH LACCASE" ISWPC,1997, pages 1-5, XP000863181 the whole document	
A	BOURBONNAIS R ET AL: "ENZYMATIC DELIGNIFICATION OF KRAFT PULP USING LACCASE AND A MEDIATOR" TAPPI JOURNAL,US,TECHNICAL ASSOCIATION OF THE PULP & PAPER INDUSTRY. ATLANTA, vol. 79, no. 6, 1 June 1996 (1996-06-01), pages 199-204, XP000640089 ISSN: 0734-1415 the whole document	
P,X	DE 197 52 666 A (IPK INST FUER PFLANZENGENETIK) 1 July 1999 (1999-07-01) the whole document	1-4, 15-17
E	WO 00 05381 A (CALGENE LLC ;SCHAAF DAVID J (US); STALKER DAVID M (US); THOMAS STE) 3 February 2000 (2000-02-03) page 4; claim 9	1-11, 13-15, 18,20-26

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/23256

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9811205 A	19-03-1998	US	5850020 A	15-12-1998
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		CN	1157636 A	20-08-1997
		EP	0767836 A	16-04-1997
		FI	965201 A	21-02-1997
		JP	10502806 T	17-03-1998
		NZ	288901 A	27-04-1998
		WO	9600290 A	04-01-1996
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WO 0005381 A	03-02-2000	US	6013860 A	11-01-2000
		EP	1017824 A	12-07-2000